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Free Energy Study of Ion Permeation through Gramicidin

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The pentadecapeptide gramicidin forms a cation-specific ion channel in membrane environment. Two conformations are known up-to-date: the head-to-head helical dimer (HD) and the intertwined double helical form (DH). These two conformations are favored depending on the specific conditions, but the biologically active form is still a matter of debate. Nevertheless, due to its small size, the gramicidin serves as an excellent ion channel model for both computational and experimental studies. In this comparative study, we focus on the energetics of single potassium ion permeation by means of the potential of mean force (PMF) for both gramicidin conformations using molecular dynamics simulations. Our results show that the DH has a significantly decreased central barrier with respect to HD, implying an increased ion conduction. The barrier to ion passage is found to be closely related to the channel flexibility. Multiple ion permeation for the DH conformation is probably facilitated due to its opposing pore water dipole moments at the pore entrances.

1 Introduction

Gramicidin A (gA) is the major component of the antibiotic gramicidin from the soil bacteria *Bacillus brevis*. Each monomer is made up of 15 alternating L- and D-amino acids capped at the two ends by a formyl group and an ethanolamine group. When dimerized, gA functions as a cation-selective transmembrane channel. The unique sequence of the gA peptide is able to adopt a wide range of conformations based on various environmental factors. Mainly, two folding motifs of gramicidin were reported in structural experimental studies, namely the single-stranded head-to-head dimer (HD) and the double-stranded helical dimer (DH). While the head-to-head dimer was believed to be the more thermodynamically stable form in the membrane, experiments demonstrated that the double-stranded dimer coexists with the single-stranded form in the membrane in certain proportion[1].

Due to its small size and well-defined channel pore, gramicidin is a popular model for studying the properties and mechanism of ion conduction. Several computational studies, mainly of the HD dimer were published recently[2]. All of them showed that the free energy of ion permeation through the HD channel contains an unexpectedly high central energy barrier and relatively weak binding sites at the two mouths of the channel. On the other hand, the DH conformation has been shown to translocate a water column at an increased rate as compared to HD[3,4]. The opposing pore water dipoles found only in DH suggested a facilitation of multiple ion passage in the double-stranded structure.[4]

In this study, we focus on the ion conduction properties of both DH and HD conformations. By employing the free energy calculation method, we construct the potential of mean force (PMF) of the ion permeation pathway. Our result show a decreased free energy barrier and an increased structural flexibility for DH as compared to HD.

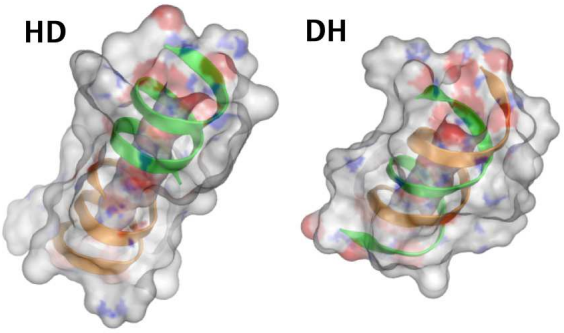

Figure 1. The two major conformations of gramicidin (PDB 1MAG for HD, 1AV2 for DH) drawn in its solvent accessible surface (water radius 1.4 Å).

2 Method

The simulation system consists of a gA (see Figure 1), 124 dimyristoylphosphatidylcholine (DMPC) lipids and 6,142 water molecules with an ionic concentration of 200 mM KCL. For the PMF of ion permeation, the ion's positions along the gramicidin channel are sampled applying the umbrella sampling technique. The PMF is then calculated by unbiasing and combining the ion density distributions of the window simulations (0.5 Å intervals) along the direction of the bilayer normal using the Weighted Histogram Analysis Method (WHAM). Molecular dynamics simulations were performed using the GROMACS package with the GROMOS53a6 protein force field and the Berger lipid force field.

3 Results

The symmetrized PMF profiles of the two gramicidin conformations show a remarkable difference in stabilizing a K^+ ion along the gramicidin channel. As shown in Figure 2, the HD profile has a large central barrier of about 46 kJ/mol. A wide shallow well is observed at the interface of the channel and the lipid headgroup region. Ion entering the channel experience a stepwise increase in free energy. Results obtained in this study for the HD conformation are in good agreement with previous free energy calculations for the barrier height[2]. In contrast, the DH profile has a much less rugged energy profile with a decreased central barrier of only 15 kJ/mol, at least a factor of three lower than for HD. Binding sites at the channel entrance as well as in the lipid interfacial region are clearly seen in the symmetrized profile. Experimentally, it was observed that gramicidin contains two symmetrically related binding sites at both ends of the channel.

Structural changes of the channel in response to the presence of a K^+ ion are e.g. reflected by changes in the pore radius. The DH conformation has a pore with a uniform radius of ≈ 1.7 Å in the ion-free state. In contrast, the pore is more narrow for the HD conformation at the channel entrances (≈ 1.0 Å). In the window simulations, drastic changes close to the channel openings were observed in DH which amounts to a reduction of about 30% in the pore radius in contrast to only 20% in HD. This reflects the high flexibility of the double-helical conformation in response to the conducting ion.

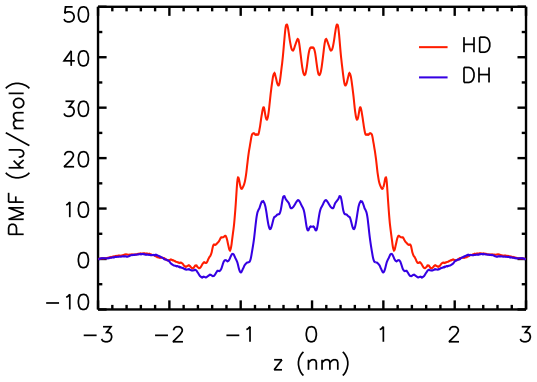

Figure 2. Compare the PMF profiles of K^+ permeating the gramicidin channel in HD and DH conformation (symmetrized).

4 Conclusion

We have shown that the double-helical dimer of gramicidin has a 3-fold decreased free energy barrier for ion permeation compared to the single-stranded dimer, possibly coupled to the high flexibility of the DH channel in coordinating the passing ion.

Acknowledgments

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